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Phenotype and individual investment in cooperative foundress associations of the fire ant, *Solenopsis invicta*

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Fire ant (*Solenopsis invicta*) queens founding a colony with unrelated nest mates potentially face a trade-off. Increased individual investment enhances worker production, colony survival, and growth. However, increased investment may reduce a queen's probability of surviving fights that invariably arise after worker eclosion. Indeed, previous studies showed that queens lose less weight (a measure of investment) when initiating colonies with cofoundresses than when alone, and that within associations the queen losing more weight is more likely to die. In this study, we tested whether queens adjust weight loss to social environment and fighting ability and whether restraining weight loss directly increases survival prospects. Experimental manipulation of colonies showed that reduced investment by queens within associations is primarily a response to the presence of a nest mate and not simply a response to per-queen brood-care demands. Differences in head width were associated with relative and combined weight loss of cofoundresses, as well as with queen survival. In contrast, the investment strategies of queens were not significantly influenced by their nest mates' initial weight. Similarly, manipulation of the queens' relative weight by feeding and exposure to contrasting social environment (queens kept alone or in groups) did not significantly affect survival. These results indicate that head width differences or correlated phenotypic attributes of fighting ability influenced both investment strategies and survival probability of queens. That queens with larger heads invested less energy into brood rearing and were more likely to survive reveals more selfish interactions among cofoundresses than has previously been assumed and casts some doubts about the idea that group selection must be invoked to account for the maintenance of cooperation in foundress associations of ants.
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Group living often is associated with behavior that provides benefits to all group members, such as defense, alertness, or care and feeding of a common brood (Pusey and Packer, 1994, 1997). However, benefits are generally not free of costs, and group members may be tempted to cheat, refraining from performing costly or dangerous tasks. This raises the question of what factors maintain stable cooperation within groups and influence the relative contribution of group members to the performance of costly tasks.

Ant foundress associations are ideally suited to study variation in individual investment and its effects on individual and group reproductive success. One feature of this system is natural variation in the degree of sociality. Queens of many species (Bourke and Franks, 1995; Hölldobler and Wilson, 1990; Rissing and Pollock, 1988) found their colonies either solitarily (haplometrosis) or in association with other queens from the same mating flight (pleometrosis). This includes the monogyne form (i.e., mature colonies contain only one queen; Ross and Fletcher, 1985a) of the fire ant *Solenopsis invicta* (Markin et al., 1972). Another feature is that individual investment can be easily estimated as weight loss because, during the founding stage in most species, queens do not forage, rearing the first workers with body reserves (fat, proteins and glycogen) they have stored before mating flight (Keller and Passera, 1989; Wheeler and Buck, 1995, 1996). Third, there is a clear effect of variation in number of foundresses both at the colony and queen level. Foundress associations have a larger first worker brood (Rissing and Pollock, 1988), faster colony growth and maturation (Vargo, 1988), and higher survival than colonies founded by single queens (Adams and Tschinkel, 1995; Balas and Adams, 1997; Bourke and Franks, 1995; Rissing and Pollock, 1988, 1991; Tschinkel, 1992; Waloff, 1957). However, these advantages of joining other queens are offset by the fact that in most species overt aggression among queens breaks out soon after worker eclosion, leading to the death or expulsion of all but one queen (Rissing and Pollock, 1988; Sommer and Hölldobler, 1995). The surviving queen secures the colony's future reproductive output (Seger, 1993). Unsuccessful queens have zero fitness, as sexual progeny are not produced at this stage, and cofoundresses are most likely unrelated in ants (Hagen et al., 1988; Sasaki, 1996; Strassmann, 1989).

Queens within associations all contribute to egg laying and brood care (Bourke and Franks, 1995). This grants an insurance to the colony but potentially creates a cheating situation for queens to selfishly restrain weight loss. Consistent with this idea, queens have been shown to lose less weight (per capita) in pleometrotic associations than in haplometrotic colonies in *S. invicta* (Markin et al., 1972; Tschinkel, 1993) and other species (Rissing and Pollock, 1988). However, the functional significance of this difference in weight loss has not been investigated. Reducing weight loss may increase an individual's relative survival probability by directly affecting fighting ability, by decreasing starvation risk, or by a queen in better condition attracting more worker attention and feeding. Alternatively, the difference in weight loss between solitary queens and queens within associations may simply reflect an adjust-

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ment to different brood care demands because foundress associations have a larger total brood size, but on a per-capita basis queens have to care for less brood within associations than do solitary queens (Tschinkel, 1993).

Previous work has shown that individual levels of weight loss within two-queen associations are correlated with both the queens' relative probability of survival and their relative contribution to worker production (Balas and Adams, 1996; Bernasconi and Keller, 1996; Bernasconi et al., 1997). Workers apparently are not able to bias the outcome of fights in favor of their mother (Balas and Adams, 1996; Bernasconi and Keller, 1996; Bernasconi et al., 1997). Two mechanisms may produce an association between differential weight loss and fighting ability: queen relative weight at the time of fights may directly influence fight outcome (Reeve and Ratnieks, 1993), or differential weight loss may be influenced by relative fighting ability, thus producing a correlation between weight loss and the probability of surviving fights.

The aim of this study was to investigate the importance of within-colony conflict for early individual investment, in particular to address (1) why queens lose less weight in pleometrotic associations than when founding a colony solitarily (Markin et al., 1972; Tschinkel, 1993) and (2) what causes differences in investment among queens within one association (Bernasconi, et al. 1997). To investigate why queens within pleometrotic associations lose less weight than haplometrotic queens, we manipulated queen number and brood care demands. To identify the causes of differential weight loss of nest-mate queens, we performed several experiments. First, we investigated whether individual investment is adjusted to differences in initial weight and in head width and whether head width explains the probability of survival of queens. Further, we fed one of two nest-mate queens during colony founding to investigate whether the relative energy stores at the time of fights directly influence relative survival probability. Finally, to test whether reduced weight loss within associations directly increases fighting ability, we competed queens whose level of investment was manipulated by previously keeping them under haplometrotic versus pleometrotic conditions.

MATERIALS AND METHODS

For all experiments, the following general methods were used. Queens were collected after mating flights from monogyne *S. invicta* populations in Georgia (on 8 June 1994 and 18 July 1995) and Florida, USA (on 31 May 1995 and 23 May 1996). They were shipped to the laboratory immediately after collection, except for 1996 queens, which were kept at 5°C for 4 days until shipment was possible. This low temperature prevented the onset of oviposition, which naturally occurs 2–3 days after mating flight (Markin, 1972; Voss and Blum, 1987). Experiments were started immediately after queens arrived to the laboratory (i.e., within 48 h of collection, except for 1996); this is referred to as day 1. On day 1 we weighed all queens (1994–1995: nearest 0.1 mg; 1996: nearest 0.01 mg) and paint-marked the thorax. Two types of artificial nests were used: petri dishes with plaster (Bernasconi and Keller, 1996) and vial nests. Vials (diam 1 cm, height 10 cm) were filled with 10 ml water, stuffed with cotton, and plugged with a cellulose stopper, forming a nest chamber. All nests were kept in a dark, ventilated chamber (28 ± 2°C, 70% ± 10% RH). We randomized assignment procedures, and nests assigned to the different treatments were randomly distributed within the rearing room. After worker eclosion, colonies were fed with homogenized beef meat every other day and observed daily until one queen was killed. Workers usually eclose on day 21, and the elimination of all but one queen as a result of fights occurs in week 4–6 from the beginning of colony founding (Markin et al., 1972; Voss and Blum, 1987).

Table 1
Colonies discarded from analysis and final sample sizes

Experiment	Response measured	Diploid males	Both queens died or early queen death	No execution up to day 60	Final sample size
1	Weight loss	5	37	—	86
2	Weight loss	—	—	—	44
3	Weight loss, survival	—	28	1	59
4	Survival	4	7	9	51
5	Survival	26	77	2	103
Total					343

Colonies for experiment 2 were selected on day 21, and replicates discarded beforehand were not considered.

In some of the experiments we combined queens from different nests (e.g., to assess survival of queens kept under different treatments). Recombined associations were given a fresh nest and an unrelated, unfamiliar brood. Queen and brood transfer do not elicit aggression in most cases when performed before worker eclosion (see Balas and Adams, 1996; Bernasconi and Keller, 1996). As queens within the same association are not statistically independent, to avoid pseudoreplication (Hurlbert, 1984) we analyzed individual data from only one queen per nest (referred to as "focal" queen), randomly chosen at the start of experiments unless specified. Other queens in the same association are referred to as "nest mates."

Because queens do not forage, individual investment can be measured as weight loss before worker eclosion. In *S. invicta* queens lose up to 50% of their initial weight within the first 3 weeks of colony founding (i.e., the time required for development of the first workers) (Balas and Adams, 1996; Bernasconi and Keller, 1996; Tschinkel, 1993). Weight loss is most likely a good indicator of the queens' investment in producing and feeding the brood because the size of the first worker brood is positively correlated with the combined weight loss of cofoundresses and with weight loss of solitary queens (Balas and Adams, 1996; Bernasconi and Keller, 1996; Tschinkel, 1993). Weight loss of individual queens is given as proportion of the initial weight (weight on day 1 unless specified). Combined weight loss of queens in two-queen associations is the total weight loss of both queens as proportion of their total initial weight.

Some queens produced diploid males. Diploid males are homozygous at the sex-determining locus, and this prevents normal development into female workers; their occurrence lowers colony survival (Ross and Fletcher, 1985b). Colonies with diploid males, where both queens died after fights or where one queen died before worker eclosion, and where queen execution occurred after day 60 or later were excluded from analysis (Table 1). Unless otherwise specified, data are given as means ± SD.

Do queens adjust weight loss to social environment and brood care demands?

To investigate whether restrained weight loss of queens within pleometrotic associations is a response to the presence of nest mates and/or an adjustment to differential brood care demands, we manipulated queen number and brood composi-

tion during the third week of colony founding. Until day 14, 128 queens were kept singly in vial nests. These queens were used as focal queens. On day 14, queens were transferred to petri-dish nests, where they were assigned to one of the following treatments ($n = 32$ each): "solitary–high brood," "solitary–low brood," "paired–high brood," and "paired–low brood." Queens in the "paired" treatments were given a nest mate taken from other two-queen associations ($n = 120$) set up on day 1 and kept under the same conditions. The "high brood" treatments simulated the per-queen brood care demands found in haplometrotic colonies; thus broods were taken from haplometrotic queens (one if the focal queen was alone and by merging two haplometrotic broods if the queen was given a nest mate). The "low brood" treatments simulated the per-queen brood care demands in pleometrotic associations. Thus, broods for this treatment group were taken from two-queen associations. If the queen was alone, it received only half a brood from a two-queen association, whereas in the "paired–low brood" treatment each experimental association received the full brood from another two-queen colony.

We weighed queens on day 21 to determine whether the treatment had an effect on weight loss. We calculated weight loss of focal queens from day 10 to 21 as the proportion of their weight on day 10. Initial weight was measured on day 10 (and not 14, when the experiment began) because of time constraints on simultaneously carrying out several experiments with large sample sizes. Before treatment, the weight of queens was homogeneous among treatment levels (ANOVA, $F_{3,124} = 0.07$, $p > .9$). For ANOVA we used Searle's weighted means method for unbalanced designs (SYSTAT, 1992).

Do queens adjust weight loss to relative initial weight?

To test whether queens adjust weight loss in response to initial weight differences between cofoundresses, we paired queens of different weight in vial nests on day 1. In half of the nests, the focal queen was given a heavier nest mate and in the other half the focal queen was given a lighter nest mate. The weight loss of focal queens exposed to a heavier versus a lighter nest mate was compared pairwise between focal queens of the same initial weight. We chose this approach (matched-pairs) because focal queens vary in initial weight, and their own weight may also influence weight loss, and because of the higher statistical power of paired tests. Thus, although focal queens were of the same initial weight (mean difference: 0.1 ± 0.4 mg, paired t test between matched colonies, $t = 1.1$, $n = 22$, $p = .27$), their nest mates differed significantly in initial weight (lighter nest mates: 14.6 ± 0.9 mg; heavier nest mates: 15.8 ± 0.6 mg, paired t test, $t = -6.9$, $n = 22$, $p < .0001$). Queens were reweighed on day 21. On day 21 there were 22 matched pairs of colonies (i.e., 44 colonies in total).

Do head width differences influence investment and survival?

To test whether head width differences of cofoundresses are correlated with investment and individual survival probability, we conducted a cross-fostering experiment on day 20 where queens were paired according to their relative weight loss during the first 20 days of colony founding. During the first 20 days of the experiment queens of the same initial weight (average difference 0.01 ± 0.12 mg) were housed pairwise in vial nests. On day 20, nest-mate queens differed by 4.1 ± 1.3 mg. The queen that had lost less weight (focal queen) was transferred to a plaster nest with another unfamiliar queen of the same initial weight (weight at day 1: paired t test: $t = -0.2$, $n = 59$, $p = .8$), but which had lost more weight than her previous nest mate until day 20. This transfer was carried out to simultaneously investigate the correlation of head width difference, differential weight loss, and their interaction on survival of the focal queen in competition with an unfamiliar nest mate. We monitored the recombined associations until workers eclosed and one of the two queens was killed. Head width was measured to the nearest micron (10 × 25 magnification; measuring table Completron CX, A. Steinmeyer, Albstadt-Ebingen, Germany) on preserved queens. In some of these colonies the head width of both queens could not be measured so that sample sizes are 43 for the analysis of head width and weight loss in early colonies and 54 for the analysis of head width and survival in recombined colonies.

We used a multiple regression with backward elimination to test whether the difference in head width and/or the average head width of the two nest-mate queens affected their combined weight loss up to day 20. Average head width and head width difference were not significantly correlated ($r = .22$, $n = 43$ queen pairs, $p = .16$). As a control, we examined whether the absolute head width of queens was correlated with their weight loss in 22 single-queen colonies. Queens for the control were collected during the same mating flight and were kept singly, but otherwise under the same conditions as the two-queen associations.

Factors affecting the probability of survival of queens in recombined associations were analyzed with multivariate logistic regression, a method suitable for a dichotomous response (whether the focal queen survived or not) and continuous regressor variables (Hosmer and Lemeshow, 1989). We simultaneously examined the difference in head width and the difference in weight on day 20 between the two queens within recombined associations. Difference in weight on day 20 resulted from differential weight loss, as queens within recombined associations were of the same initial weight. Both head width and weight difference were entered as signed differences in the model (focal nest mate).

Differential feeding and survival

Pairs of queens of the same initial weight ($n = 74$) were placed on day 1 in petri-dish nests. To manipulate the queens' relative weight near worker eclosion, we fed only one of the queens in a pair. Both queens were isolated for 8 h, but only the treatment queen received food. In a first experiment ($n = 43$ queen pairs), the queens were simultaneously transferred to an empty nest and the treatment queen received half a mealworm; this was done twice (on day 17 and 18). In a second experiment ($n = 31$ queen pairs), the treatment queen was given a queen abdomen once on day 12 and once on day 17. Consumption of dead queens occurs in foundress associations (Tschinkel, 1993; Bernasconi G, unpublished data); however, queens during this stage have to rely on own body reserves, and for this reason we fed queens only twice. Queen abdomens were obtained by freezing healthy looking queens on day 1. In this experiment, only one queen was removed from the nest, the other remaining in the nest with the brood. In half of the associations we removed first the treatment queen on day 12 and the control queen on day 17. The reversed order of removal was followed for the other half of replicates. All queens were weighed before (day 7 in experiment 1, day 17 in experiment 2) and after treatment (day 19 in both experiments). For analysis, there were 51 associations, 26 from the first, and 25 from the second experiment.

Survival of competing haplometrotic and pleometrotic queens

To test whether differential weight loss of queens influences their survival likelihood, we paired queens that were previously kept under haplometrotic and pleometrotic conditions. If relative weight of queens is the prime factor determining survival probability, queens kept haplometrotically should be less likely to survive than queens kept in pleometrotic associations when two such queens compete within the same colony because queens lose more weight in haplometrotic colonies

(Markin et al., 1972; Tschinkel, 1993; this study). Competition between haplometrotic and pleometrotic queens may occur under natural conditions, as some queens leave their nests and try to usurp a neighboring one (Adams and Tschinkel, 1995; Balas and Adams, 1997).

On day 1 we isolated 252 queens (haplometrotic treatment) and with another 360 queens we set up 90 associations with four queens each (pleometrotic treatment), all kept in plaster nests. We assigned more queens to the pleometrotic treatment because the probability that associations contain diploid males increases with queen number, and therefore we expected to discard more colonies in this treatment. The four queens in pleometrotic associations were of the same initial weight. On day 10 we constructed 104 recombined queen pairs, each containing 1 queen from the haplometrotic treatment and 1 queen from the pleometrotic treatment. To set up these queen pairs we selected four haplometrotic queens of same initial weight as the queens in a given pleometrotic association (maximum difference: 0.2 mg) and mixed the brood of these eight queens (four haplometrotic and four queens from one pleometrotic association). The brood was distributed evenly among the recombined queen pairs. The same procedure was repeated on day 20 to construct another 104 recombined queen pairs. Only intact pleometrotic associations where all queens were still alive (on day 10 or 20, respectively) were used to build recombined queen pairs. Recombined queen pairs were observed until one queen was killed. Of the final sample size of 103 associations, 46 were built on day 10 and 57 on day 20. Duration of treatment (10 or 20 days) was not significantly associated with the outcome of fights (log likelihood-ratio heterogeneity test, $G < 0.01$, df = 1, $p > .90$), thus we pooled the data.

RESULTS

Do queens adjust weight loss to social environment and brood care demands?

A two-way ANOVA showed that queens exposed to the presence of a nest mate during the third week of colony founding lost significantly less weight (21% ± 9%, $n = 38$, weight loss as percentage of weight on day 10) than queens not exposed to a nest mate (26% ± 8%, $n = 48$; $F_{1, 82} = 6.79$, $p = .01$). In contrast, neither brood composition ($F_{1, 82} = 0.31$, $p = .58$) nor the two-way interaction ($F_{1, 82} = 0.20$, $p = .65$) were significant (Figure 1).

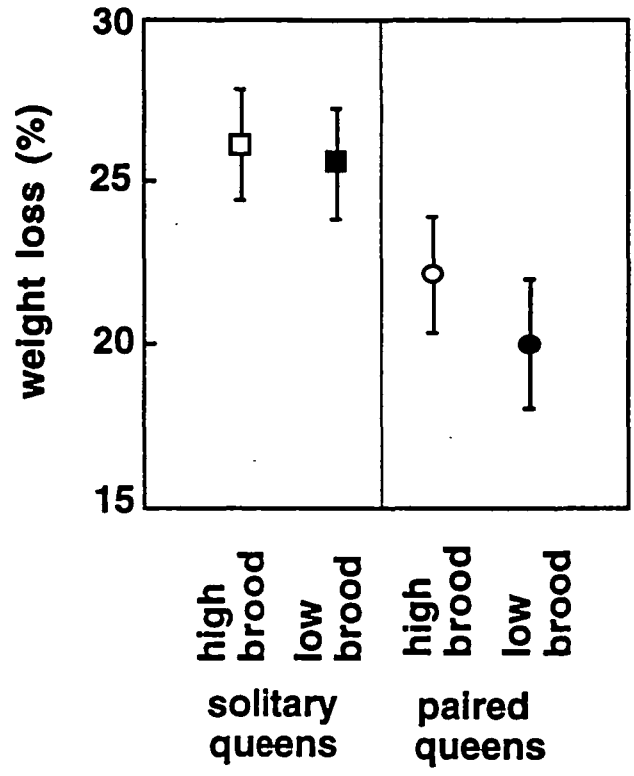

Figure 1
Relative individual weight loss (% of weight before treatment; mean ± SE) of focal queens between day 10 and day 21. Treatment groups represent different social environments and brood composition. Solitary queens: high per-queen brood (open squares, $n = 24$); low per-queen brood, (filled squares, $n = 24$). Paired queens: high per-queen brood (open circles, $n = 21$); low per-queen brood (filled circles, $n = 17$).

Do queens adjust weight loss to relative initial weight?

There was no significant difference in weight loss between queens exposed to a heavier nest mate (38.9% ± 8.6%, $n = 22$) and queens exposed to a lighter nest mate (41.1% ± 6.2%, $n = 22$; paired t test: $t = 0.81$, $p > .40$). A multiple regression showed that neither absolute initial weight of queens nor the initial weight difference between queens within associations significantly influenced their combined weight loss (multiple regression $F_{2, 41} = 1.3$, $p = .28$, partial test for average initial weight: $t = 1.6$, $p = .12$; partial test for unsigned weight difference: $t = 0.5$, $p = .61$).

Do head width differences influence investment and survival?

Individual weight loss was significantly associated with the relative head width of queens within the same association. The queens losing less weight within each association had a significantly larger head (1.41 ± 0.04 mm) than their nest mates (1.39 ± 0.03 mm; paired t test, $t = 3.5$, $n = 43$, $p = .001$). Head width was not significantly correlated with the weight of queens on day 1 (i.e., just after the mating flight; $r_s = .08$, $n = 108$, $p > .50$).

The association between head width and weight loss principally resulted from the differential response to head width difference by the larger-headed queen. The amount of weight investment by the queen that lost less weight within a pair was significantly negatively correlated with the difference in head width between her and her nest mate ($r_s = -.41$, $n = 43$, $p < .01$). In contrast, there was no significant relationship between individual weight loss and head width difference for the queens that lost more weight within pairs ($r_s = -.09$, $n = 43$, $p > .50$). For control queens kept alone, there was no significant correlation between weight loss and head width ($r_s = .02$, $n = 22$, $p > .90$).

A multiple regression also revealed that the combined weight loss of queens up to day 20 was negatively correlated with the head width difference between nest mates ($F_{1, 41} = 4.86$, $p = .03$; Figure 2), but not with their average head width ($F_{1, 41} = .78$, $p = .38$).

In recombined associations, the focal queen was both heavier (difference on day 20: 3.1 ± 1.1 mg, paired t test $= -10.2$, $n = 59$, $p < .01$) and had a significantly larger head (paired t test, $t = 2.4$, $n = 54$, $p = .02$) than her nest mate. The weight difference was expected, as focal queens were the queens that had lost less weight up to day 20 in a two-queen association and their new nest mates were queens that had lost more weight in a two-queen association of the same type. The significant difference in head width reflects the tendency of larger-headed queens to lose less weight (see above).

The focal queen survived in 37 (63%) out of 59 associations. Multivariate logistic regression revealed that head width difference rather than weight difference is the important factor influencing probability of survival in recombined associa-

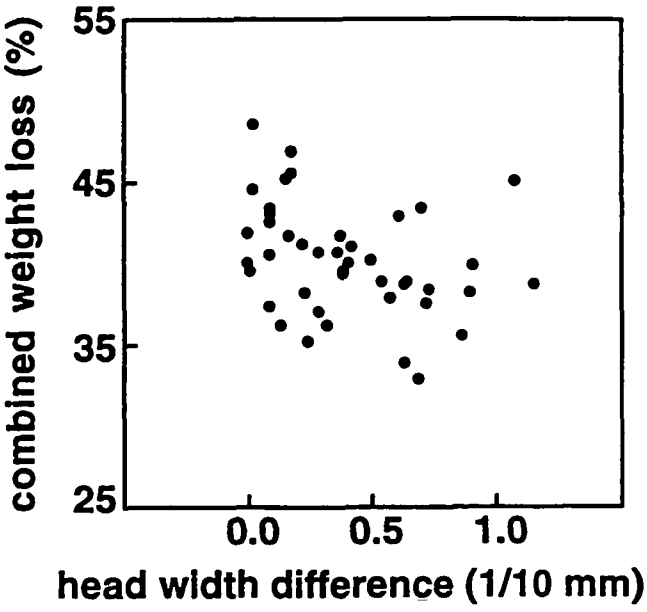

Figure 2
Combined investment of the focus queen and her nest mate from day 1 to day 20 plotted against the absolute value of the head width difference between the focal queen and her nest mate. Investment is measured as weight loss (% of the initial weight; queens within two-queen associations of the same initial weight, $n = 43$).

tions (Figure 3). This analysis shows that head width difference was significantly associated with the probability of survival of the focal queen (log-likelihood ratio test: deviance difference to null model: 7.8, df = 1, $p < .01$), while neither the weight difference nor the interaction between weight difference and head width difference was significant (Table 2).

Differential feeding and survival

Queens that were given food were not significantly more likely to survive than control queens both in the first (10 out 26 associations; binomial test: $p = .33$) and in the second experiment (10 out 25 associations; binomial test: $p = .42$). In fact, there was a slightly higher mortality of queens that received food. The lack of a significant effect cannot be ascribed to a lack of effect of our treatment in the first experiment, where queens that were given mealworms were significantly heavier than control queens after food was provided (Table 3). In the second experiment, where queens were first fed early during colony founding (day 12), there was no significant difference between queens 1 week later (table 3).

Survival of competing haplometrotic and pleometrotic queens

As expected, queens in the haplometrotic treatment lost significantly more weight than queens in the pleometrotic treatment (nested ANOVA within blocks of four pleometrotic queens and their haplometrotic nest mates of the same initial weight; effect of treatment: $F_{4,200} = 14.8$, $p < .001$), and this difference increased with treatment duration (10 versus 20 days: $F_{1,200} = 485.6$, $p < .001$), resulting in pleometrotic queens being heavier on the day of joining than haplometrotic queens. However, pleometrotic queens were not more likely to survive than haplometrotic queens. In fact, pleometrotic queens survived in less than half of the colonies (47 out of 103 colonies, 46%; binomial test, $z = -0.79$, $p > .40$), indicating that the greater weight of pleometrotic queens did not provide an advantage to these queens when in competition with queens that were previously kept under haplometrotic conditions.

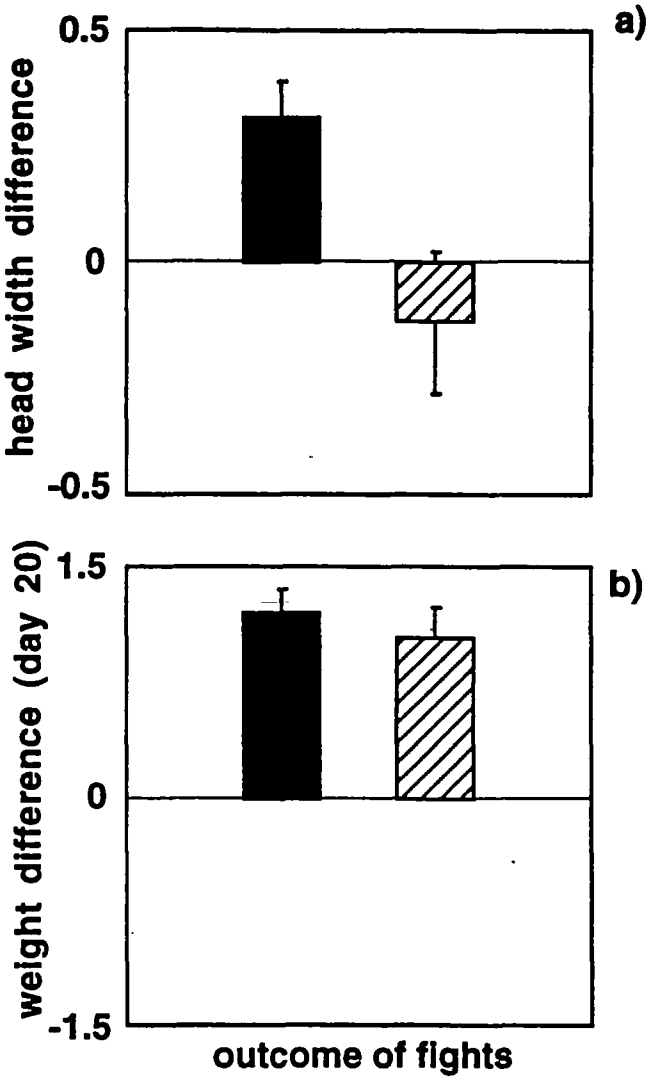

Figure 3
(a) Head width difference (mean ± SE; 1/10 mm; focal : nest mate) in associations where the focal queen survived (black bars, $n = 33$) and associations, where the focal queen was killed (striped bars, $n = 21$; Mann-Whitney U test, $U_{min} = 201.5$, $p = .01$). (b) Weight difference (mg) between queens in associations where the focal queen survived (black bars) and where the nest mate survived (striped bars; independent t test, $t = 0.68$, $p > .50$). Queens within associations were of the same initial weight.

DISCUSSION

Our results have important implications for the issue of maintenance of cooperation within genetically heterogeneous groups, such as the apparently altruistic behavior of unrelated ant cofoundresses during early colony founding. We investigated whether two observed differences in investment levels, (1) between queens within associations and solitary queens, and (2) among queens within the same association, reveal within-group conflicts. We focused on investment levels, because they enhance colony survivorship, but have potential costs to individual queens.

Our first experiment shows that the lower individual weight loss of queens within pleometrotic associations is primarily or only a response to the presence of a nest mate and not simply due to differences in brood composition between haplometrotic and pleometrotic colonies. The lower weight loss of queens in pleometrotic associations may stem either from a

Table 2
Multiple logistic regression for the determinants of survival of focal queens in competition with an unfamiliar nest mate

Model	Deviance	Deviance difference	df	p
Constant (null model)	72.2		53	
Constant + *head width difference*	64.3	7.9	52	<.01
Constant + head width difference + *weight difference*	64.3	<0.1	51	ns
Constant + head width difference (A) + weight difference (B) + *interaction (A*B)*	64.2	0.1	50	ns

Minimum adequate model: y (logit) = 0.28 (±0.31) + 1.48 (±0.59) * head width difference (goodness-of-fit test: $p > .10$). p values = log-likelihood ratio test for the effect of removing the variable in italic from the model; head width difference in 1/10 mm.

lower investment in brood care and/or from egg cannibalism. Evidence suggests that egg cannibalism may play a role because it occurs in *S. invicta* associations (Tschinkel, 1993), but not when queens are alone (Voss and Blum, 1987).

Because colony productivity is positively correlated with the total investment of nest mate queens (Balas and Adams, 1996; Bernasconi and Keller, 1996; Tschinkel, 1993), the reduced individual investment by queens in pleometrotic associations is likely to incur costs to the colony. Indeed, the number of workers produced per queen is highest for solitary queens and decreases with increasing queen number in pleometrotic associations (Tschinkel, 1993). Reduced individual investment might be explained if queens, by losing less weight, are heavier at the time of fights and this increases their chances of surviving fights. Previous experiments indeed showed correlationally that differential weight loss of queens during colony founding is associated with survival (Balas and Adams, 1996; Bernasconi and Keller, 1996). However, all the experiments conducted in this study indicate that differential weight loss of nest mates is probably not directly influencing survival but most likely reflects phenotypic asymmetries between nest mate queens that influence both differential weight loss and survival likelihood.

Head width differences between queens influenced individual and combined weight loss during colony founding and were significantly correlated with the survival probability of queens competing against an unfamiliar nest mate. Weight loss was lower the greater the difference between queens, independently of absolute queen head width. This suggests that queens adjust their investment to relative fighting ability. In contrast, weight differences at worker eclosion did not significantly influence survival. That weight and the amount of fat and energy reserves at the time of worker eclosion might not be the main factor affecting the outcome of fights is further supported by the feeding experiments. The queens that received food during colony founding were no more likely to survive fights than queens that received no food. Feeding may fail to manipulate queen weight if queens engage in trophallaxis (exchange of liquid food; Vargo EL, personal communication), as may be suggested by the lack of significant difference between queens after feeding in one of the feeding treatments. This does not apply to the experiment where pleometrotic queens were in competition with haplometrotic queens. Pleometrotic queens lost less weight and were thus significantly heavier at the time of fight outbreak, but they did not survive more frequently than haplometrotic queens. It is important to point out that our study addresses the effect of weight differences near fight outbreak as they result from differential weight loss. This is distinct from the situation where late differences result from initial differences (Balas and Adams, 1996; Bernasconi and Keller, 1996). However, the experiment where queens were paired with queens of lower or higher weight failed to demonstrate an adjustment of individual investment to initial weight difference. Whatever the weight of their nest mates, focal queens lost the same amount of weight.

Altogether these data indicate that head width differences among nest mates, and/or other phenotypic differences associated with head width, influence the investment strategies of queens and, ultimately, their probability of survival. A possible advantage for larger queens, and presumably superior phenotypes, to losing less weight during colony founding is lower risk of starvation before worker eclosion (Pollock and Rissing, 1988). During colony founding queens are under considerable physiological stress because they do not forage (Wheeler and Buck, 1996). Hence, the queens losing less weight might be more likely to survive periods of food shortage before or just after the emergence of the first workers. Consistent with this hypothesis, queen survival has been shown to be higher in pleometrotic than haplometrotic laboratory colonies of the ant *Lasius flavus* (Waloff, 1957). Queen starvation also likely occurs in *L. pallitarsis* (Nonacs, 1990). Alternatively, queens may benefit from losing less weight if body reserves (as affected by differential weight loss) determine queen fecundity at the time of the first worker eclosion and if workers preferentially feed the more fecund queen. There is significant observational evidence that this form of preferential feeding occurs in *Lasius niger* (Sommer and Hölldobler, 1995).

A correlation between body size and dominance hierarchies has been demonstrated in several wasp species (Reeve, 1991; Turillazzi and Pardi, 1977). The finding that initial phenotypic difference among founding queens influences the probability of survival raises the question of why individuals with low fighting ability cooperate with superior queens. If queens are unrelated, as presumably is generally the case for ant cofoundresses (Strassmann, 1989), weaker competitors should

Table 3
Weight before and after feeding for treatment and control queens

		Weight (mg)			
		Fed	Control	t	p
Mealworms, day 17 and 18 (n = 26)	Before (day 17)	10.3 ± 1.5	10.6 ± 2.0	−0.6	>.5
	After (day 19)	12.4 ± 2.1	10.4 ± 1.4	3.8	<.001
Queen abdomens, day 12 and 17 (n = 25)	Before (day 7)	12.9 ± 1.1	13.0 ± 1.3	−0.6	>.5
	After (day 19)	10.6 ± 1.2	10.3 ± 1.1	1.07	.3

t = paired t-test.

not cooperate unless they have some probability of survival and produce reproductive offspring. Thus, Nonacs (1989) suggested that ant queens should show conditional joining behavior and discriminate nest mates by relative competitive ability. To our knowledge the possibility that queens may choose cofoundresses in a nonrandom manner has not yet been investigated in *S. invicta*. Although head width differences between nest mates significantly influenced both investment and survival, it is noteworthy that there was only a relatively weak association between these parameters (i.e., head width asymmetry is not entirely divisive; sensu Grafen, 1987). This might be necessary to maintain cooperation between cofoundresses because queens will help perform costly tasks such as brood rearing only if they have a chance of surviving. In their game-theoretical analysis of the evolution of fatal fighting, Enquist and Leimar (1990) emphasized that the critical factor for the evolution of fatal fighting is the relationship between the contested resource and the lifetime utility if an individual does not gain the resource (called "value of the future"). If the future is of low value compared to the contested resource, individuals may fight even if their probability of winning against an opponent is less than .5. Hence, a queen with a smaller head, and presumably lower fighting ability, may still benefit by feeding the common brood if her chances of succeeding (considering also colony survival) are higher than as a solitary foundress.

Early colony founding by ant associations has frequently been proposed as an example of group selection because unrelated queens cooperate in an apparently unselfish manner to raise a large common brood that increases colony survivorship (Dugatkin et al., 1992; Mesterton-Gibbons and Dugatkin, 1992; Wilson, 1990). The outbreak of fatal fights indicates reproductive conflicts among cofoundresses but has been ascribed to food competition after workers start foraging (Rissing and Pollock, 1988). Our study shows that the lower individual weight loss by queens within associations compared to solitary queens (Markin et al., 1972; Tschinkel, 1993) cannot be explained by differences in brood care demands. Rather, queens in cooperative associations invest less energy in raising the brood. Moreover, investment of cofoundresses is adjusted to head width difference, a phenotypic trait associated with fighting ability. Larger queens both invest less energy in rearing the brood and are more likely to survive. Genetic analyses also showed that the queen that invests the least energy in brood production achieves a greater share of maternity in the first worker brood and is most likely to survive (Bernasconi et al., 1997). Together these results indicate that in *S. invicta* fighting ability most likely plays a major role in queen–queen conflicts, both for the outcome of fights and in regulating individual investment levels long before aggression becomes overt. Thus, long before the outbreak of overt aggression, conflicts among individual queens within associations become apparent. The occurrrence of such conflicts and the possibility that they may account for the apparently altruistic acts by cofoundresses have been largely neglected in previous studies. In the leaf-cutter ant, *Acromyrmex versicolor*, for example, one of the cofoundresses becomes a foraging specialist before the emergence of the workers (Rissing et al., 1989). It was thought that this decision is not a coercive one, but foraging specialization may stem from subtle and difficult-to-observe interactions among cofoundresses. Rissing et al. (1996) indeed found some evidence that the forager may be punished by her nest mates if she discontinues food gathering. These data indicate that group selection need not be invoked to account for the apparently altruistic behavior of unrelated ant foundresses.

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REFERENCES

Adams ES, Tschinkel WR, 1995. Effects of foundress number on brood raids and queen survival in the fire ant *Solenopsis invicta*. Behav Ecol Sociobiol 37:233–242.

Balas MT, Adams ES, 1996. The dissolution of cooperative groups: mechanisms of queen mortality in incipient fire ant colonies. Behav Ecol Sociobiol 38:391–399.

Balas MT, Adams ES, 1997. Intraspecific usurpation of incipient fire ant colonies. Behav Ecol 8:99–103.

Bernasconi G, Keller L, 1996. Reproductive conflicts in cooperative associations of fire ant queens, *Solenopsis invicta*. Proc R Soc Lond B 263:509–513.

Bernasconi G, Krieger MJB, Keller L, 1997. Unequal partitioning of reproduction and investment between cooperating queens in the fire ant, *Solenopsis invicta*, as revealed by microsatellites. Proc R Soc Lond B 264:1331–1336.

Bourke AFG, Franks NR, 1995. Social evolution in ants. Princeton, New Jersey: Princeton University Press.

Dugatkin LA, Mesterton-Gibbons M, Houston IA, 1992. Beyond the prisoner's dilemma: toward models to discriminate among mechanisms of cooperation in nature. Trends Ecol Evol 7:202–205.

Enquist M, Leimar O, 1990. The evolution of fatal fighting. Anim Behav 39:1–9.

Grafen A, 1987. The logic of divisively asymmetric contests: respect for ownership and the desperado effect. Anim Behav 35:462–467.

Hagen RH, Smith DR, Rissing SW, 1988. Genetic relatedness among co-foundresses of two desert ants, *Veromessor pergandei* and *Acromyrmex versicolor* (Hymenoptera: Formicidae). Psyche 95:191–202.

Hölldobler B, Wilson EO, 1990. The ants. Berlin: Springer.

Hosmer D, Lemeshow S, 1989. Applied logistic regression. New York: Wiley.

Hurlbert SH, 1984. Pseudoreplication and the design of ecological field experiments. Ecol Mon 54:187–211.

Keller L, Passera L, 1989. Size and fat content of gynes in relation to the mode of colony founding in ants (Hymenoptera; Formicidae). Oecologia 80:236–240.

Markin GP, Collins HL, Dillier JH, 1972. Colony founding by queens of the red imported fire ant, *Solenopsis invicta*. Ann Entomol Soc Am 65:1053–1058.

Mesterton-Gibbons M, Dugatkin LA, 1992. Cooperation among unrelated individuals: evolutionary factors. Q Rev Biol 67: 267–281.

Nonacs P, 1989. Competition and kin discrimination in colony founding by social Hymenoptera. Evol Ecol 3:221–235.

Nonacs P, 1990. Size and kinship affect success of co-founding *Lasius pallitarsis* queens. Psyche 97:217–228.

Pollock GW, Rissing SW, 1988. Social competition under mandatory group life. In: The ecology of social behavior (Slobodchikoff CN, ed). San Diego, California: Academic Press; 315–334.

Pusey A, Packer C, 1994. Non-offspring nursing in social carnivores: minimizing the costs. Behav Ecol 5:362–374.

Pusey A, Packer C, 1997. The ecology of relationships. In: Behavioural ecology, 4th ed (Krebs J, Davies, N, eds). Oxford: Blackwell; 254–283.

Reeve HK, 1991. Polistes. In: The social biology of wasps (Ross KG, Matthews RW, eds). Ithaca, New York: Cornell University Press; 99–148.

Reeve HK, Ratnieks FLW, 1993. Queen-queen conflicts in polygynous societies: mutual tolerance and reproductive skew. In: Queen number and sociality in insects (Keller L, ed). Oxford: Oxford University Press; 45–85.

Rissing SW, Pollock GW, 1988. Pleometrosis and polygyny in ants. In: Interindividual behavioral variability in social insects (Jeanne RJ, ed). Boulder, Colorado: Westview Press; 179–222.

Rissing SW, Pollock GB, 1991. An experimental analysis of pleometrotic advantage in the desert seed-harvester ant *Messor pergandei* (Hymenoptera: Formicidae). Insectes Soc 38:205–211.

Rissing SW, Pollock GW, Higgins MR, 1996. Fate of ant foundress associations containing "cheaters". Naturwissenschaften 83:182–185.

Rissing SW, Pollock GW, Higgins MR, Hagen RH, Smith DR, 1989. Foraging specialization without relatedness or dominance among cofounding queens. Nature 338:420–422.

Ross KG, Fletcher DJC, 1985a. Comparative study of genetic and social structure in two forms of the fire ant *Solenopsis invicta* (Hymenoptera: Formicidae). Behav Ecol Sociobiol 17:349–356.

Ross KG, Fletcher DJC, 1985b. Genetic origin of male diploidy in the fire ant, *Solenopsis invicta*, and its evolutionary significance. Evolution 39:888–903.

Sasaki K, Satoh T, Obara Y, 1996. Cooperative foundation of colonies by unrelated foundresses in the ant *Polyrhachis moesta*. Insectes Soc 43:217–226.

Seger J, 1993. Opportunities and pitfalls in co-operative reproduction. In: Queen number and sociality in insects, (Keller L, ed). Oxford: Oxford University Press; 1–15.

Sommer K, Hölldobler B, 1995. Colony founding by queen association and determinants of reduction in queen number in the ant *Lasius niger*. Anim Behav 50:287–294.

Strassmann JE, 1989. Altruism and relatedness at colony foundation in social insects. Trends Ecol Evol 4:371–374.

SYSTAT, 1992. Statistics, version 5.2.1. Evanston, Illinois: SYSTAT Inc.

Tschinkel WR, 1992. Brood raiding and the population dynamics of founding and incipient colonies of the fire ant *Solenopsis invicta*. Ecol Entomol 17:179–188.

Tschinkel WR, 1993. Resource allocation, brood production and cannibalism during colony founding in the fire ant, *Solenopsis invicta*. Behav Ecol Sociobiol 33:209–223.

Turillazzi S, Pardi L, 1977. Body size and hierarchy in polygynic nests of *Polistes gallicus* (L.) (Hymenoptera: Vespidae). Monit Zool Ital 11:101–112.

Vargo EL, 1988. Effect of pleometrosis and colony size on the production of sexuals in monogyne colonies of the fire ant *Solenopsis invicta*. In: Advances in myrmecology (Trager JC, ed). Leiden: Brill; 217–225.

Voss SH, Blum MS, 1987. Trophic and embryonated egg production in founding colonies of the fire ant *Solenopsis invicta* (Hymenoptera: Formicidae). Sociobiology 13:271–278.

Waloff N, 1957. The effect of the number of queens of the ant *Lasius flavus* (Fab.) (Hymenoptera: Formicidae) on their survival and on the rate of development of the first brood. Insect Soc 4:391–408.

Wheeler DE, Buck N, 1995. Storage proteins in ants during development and colony founding. J. Insect Physiol 41:885–894.

Wheeler DE, Buck N, 1996. Depletion of reserves in ant queens during claustral colony founding. Insect Soc 43:297–302.

Wilson DS, 1990. Weak altruism, strong group selection. Oikos 59: 135–140.